Euphytica **138:** 107–112, 2004.

Performance of intersubspecific alfalfa hybrids in sward versus space planted plots

Heathcliffe Riday[1] & E. Charles Brummer[2]
[1]*US Dairy Forage Research Center, US DA-ARS, Madison WI 53706, U.S.A.;* [2]*Raymond F. Baker Center for Plant Breeding, Department of Agronomy, Iowa State University, Ames, IA 50011, U.S.A.;*
*(*author for correspondence: e-mail: riday@wisc.edu)*

Received 21 October 2003; accepted 20 April 2004

Key words: alfalfa, biomass, falcata, heterosis, plot type

Summary

Intersubspecific hybrids between *Medicago sativa* subsp. *sativa* and subsp. *falcata* show biomass yield heterosis in space planted plots. Relative biomass yield of alfalfa is known to differ in space planted versus sward plots, although the magnitude of the difference is variable. The objective of this study was to determine if and how much of the biomass yield heterosis observed in space planted alfalfa hybrids was retained in sward planted plots. Two falcata genotypes (WISFAL-6 and PI502453-1) and three elite sativa genotypes were crossed in a diallel mating design. Progeny were transplanted into space-planted plots and harvested three times per year or drilled into sward plots harvested four times per year. Hybrids of WISFAL-6 with the sativa genotypes produced as much first harvest biomass yield as intra-sativa hybrids in space-planted plots and more in sward plots. Yield in subsequent harvests was lower than intra-sativa hybrids in sward plots only. Hybrids of PI502453-1 with sativa were generally lower yielding than intra-sativa crosses. A moderate correlation was observed between biomass yield in space planted and sward plots. Heterosis expression in swards was lower than that in spaced planted nurseries for progeny of both falcata genotypes. The increased plant-to-plant competition in swards is the likely cause of the loss of heterosis.

Introduction

Alfalfa (*Medicago sativa* L.) grown commercially for hay or pasture is cultivated in densely planted swards. In contrast, many breeding and experimental procedures are necessarily conducted under space planted conditions, due to limitations in seed or to the difficulty of evaluating individual plants in densely planted plots. The relative biomass yield performance of alfalfa germplasm differs in space planted versus sward plots, although the magnitude of the differences varies among experiments (Tysdal & Kiesselbach, 1939; Pearson and Elling, 1961; Davis & Reusch, 1964). Tysdal & Kiesselbach (1939) found relative yield to be equivalent between space-planted and sward plots, although they noted that space-planted plots were "somewhat less exact." By contrast, other studies have found moderate ($r = 0.62$; Pearson & Elling, 1961) to poor ($r = 0.32$; Davis & Reusch, 1964) correlations between space-planted and drilled (i.e. sward) plots for yield.

In addition, clonal performance of parents *per se* grown in space-planted nurseries is a poor predictor of specific or general combining ability for testcross progeny in space-planted or sward plots (Theurer & Elling, 1964; Evans et al., 1966; & Elliot et al., 1972). On the basis of these results, progeny tests of potential parental clones must be conducted in swards to obtain combining ability information relevant for commercial cultivars.

Morphological traits can vary considerably between plot types; crown size is particularly affected (Tysdal & Kiesselbach, 1939; David & Reusch, 1964). Correlations between morphological yield components and biomass yield differed in sward versus space planted plots, and therefore morphological measurements made on the latter plot type were poor predictors of yield in the former (Rumbaugh, 1963; Miller et al., 1969).

In general, falcata germplasm and, to a lesser extent, sativa-falcata hybrids have a more decumbent growth habit than commercially cultivated sativa germplasm (Riday & Brummer, 2002b). Another potential weakness of falcata germplasm and sativa-falcata hybrids is their slower regrowth after harvest (Riday & Brummer, 2002b, 2003b). In Iowa and surrounding states, high producing dairy operations typically harvest alfalfa four times per year. Our previous studies of sativa-falcata hybrids were harvested three times per year (Riday & Brummer, 2002a, 2003a).

Intersubspecific hybrids between *M. sativa* subsp. *sativa* and subsp. *falcata* show biomass yield heterosis (Riday & Brummer, 2002a, 2003a). In these studies, heterosis was measured in plots with plants spaced 30 cm apart within ten plant rows, which were separated by 90 cm (Riday & Brummer, 2002a) or within paired eight plant rows 30 cm apart with adjacent plots separated by 90 cm (Riday & Brummer, 2003a). We suspected that the decumbent growth habit and slower regrowth rate of the sativa-falcata hybrid progeny compared with the elite sativa germplasm used as parents would lead to a yield penalty when grown in sward plots.

The objective of this experiment was to test the hypotheses (1) that the relationship among sativa-falcata, sativa-sativa, and falcata-falcata hybrids for biomass yield *per se* would differ under sward and space planted conditions and (2) that sativa-falcata hybrids would express less heterosis in sward than space planted evaluations.

Materials and methods

Plant material

Three elite sativa (ABI-408, C96-514, and RP-93-377) and two falcata (WISFAL-6 and PI502453-1) genotypes were chosen for this study. These same five genotypes are a subset of those previously evaluated in spaced planted trials by Riday & Brummer (2002a). Both falcata genotypes are from improved populations, albeit populations with less historical breeding activity than those from which the sativa genotypes were derived. The five selected parents were crossed in a half-diallel single-cross mating design. Due to the large amount of seed needed for sward plots, the crosses were unemasculated. Seed used in the space-planted plots reported previously (Riday & Brummer, 2002a) was from emasculated crosses. Three check cultivars ('5454', 'Innovator +Z', and 'Vernal') were included in both the spaced and sward plot trials for a total of 10 cross entries and 3 check entries.

Space-planted plots

Details of the spaced-planted plots were described previously (Riday & Brummer, 2002a). Briefly, seeds were planted in the greenhouse, and seedlings transplanted at the Agronomy and Agricultural Engineering Research Farm west of Ames, IA in a Nicollet loam soil (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) on 20 May 1998. Ten plants per plot were planted 30 cm apart within rows spaced 90 cm apart. Entries were separated by 60 cm within rows. Biomass yield on a dry matter basis was measured during three years post-establishment, with three harvests per year (27 May 1999, 7 July 1999, and 1 September 1999, 27 May 2000, 16 July 2000, and 1 September 2000, and 13 June 2001, 30 July 2001, and 14 September 2001). Wet weights were taken and adjusted to a dry matter basis. On the basis of plant counts in each plot, biomass yield was calculated on a g plant^{-1} basis.

Sward plots

Sward plots were planted at the Agronomy and Agricultural Engineering Research Farm west of Ames, IA on 1 Aug 1999. The plot design was a quadruple α-lattice, with 4 plots in each of 6 incomplete blocks, for 96 total plots. Additional replication of some entries and filler plots were included to complete the design. In each plot, three rows 90 cm long and 15 cm apart were drilled with 75 seeds per row. Plots were spaced 30 cm apart side-to-side and were separated end-to-end from an alfalfa border by 30 cm. The entire plot area was surrounded by an alfalfa border. Biomass yield on dry matter basis was measured during three post-establishment years over four harvests per year (30 May 2000, 5 July 2000, 10 August 2000, and 13 September 2000, 5 June 2001, 15 July 2001, 8 August 2001, and 13 September 2001, and 27 May 2002, 11 July 2002, 2 August 2002, and 17 September 2002). Wet weights were obtained using a flail type harvester. After adjusting for moisture content, total plot dry matter biomass yield in Mg ha^{-1} was calculated.

Data analysis

Within each plot type (i.e. space and sward) for each harvest-year combination, least squares means were calculated for each entry to eliminate incomplete block and replication effects using PROC MIXED of SAS software package (SAS, 2000). Total yearly yield, first harvest yield, and subsequent harvest yield (i.e. total yearly yield excluding first harvest yield) were evaluated. Entry mean comparisons within plot type for the three yield variables were made assuming that both years (i.e. number of years post-establishment) and entries were fixed effects. Plot types were compared using correlations.

Our primary interest was to compare heterosis levels between plot types. Therefore, for each yield variable, we calculated a heterosis percentage for each of the six sativa-falcata entries for each plot-type during each year. Heterosis percentage was estimated as:

$$\text{Heterosis (\%)} = \frac{SF - \left(\dfrac{\overline{SS} + \overline{FF}}{2}\right)}{\left(\dfrac{\overline{SS} + \overline{FF}}{2}\right)} \tag{1}$$

where, SF = sativa by falcata cross entry [1 to 6]; $\overline{SS}$ = mean of all sativa by sativa cross entries; $\overline{FF}$ = mean of all falcata by falcata cross entries.

Comparisons among mean heterosis values for each of the three yield variables were computed assuming plot type, entries, falcata parent and years as fixed effects and sativa parents as a random effect.

Results and discussion

The sward trials reported here were grown from seed produced from unemasculated crosses, unlike the emasculated crosses used to produce seed for the space planted trials (Riday & Brummer, 2002a). Although this could bias the results presented below, we do not believe the emasculation difference had a major effect for two reasons. First, numerous studies in alfalfa have shown that pollen tubes from self pollen have both slower growth and higher penetration failure than foreign pollen, leading to a low percentage of self fertilization when foreign pollen is present (Viands et al., 1988). Second, even if some self-fertilized seed resulted from our crossing for the sward trials, the resulting S_1 plants would likely be at a competitive disadvantage in sward plots, as the more vigorous hybrid plants outcompeted them during sward development over time (Viands et al., 1988).

The parents selected for this experiment are a subset of those evaluated previously (Riday & Brummer, 2002a). The WISFAL genotype was more erect and had slightly faster regrowth than the PI502453 genotype, but both falcata genotypes were more decumbent and had slower regrowth than the three sativa genotypes (Riday & Brummer, 2002b, 2003b).

In sward plots, sativa x falcata crosses (SFC) were intermediate to sativa x sativa crosses (SSC) and falcata x falcata crosses (FFC) for total yearly yield (Table 1). This is attributable to the poor performance of PI502453-1 x sativa crosses; WISFAL-6 crosses were equivalent to SSC. During first harvest, SSC and SFC were equivalent and superior to FFC (Table 1). WISFAL-6 x sativa crosses outyielded SSC during first harvest. During subsequent harvests, SSC outyielded SFC (both WISFAL-6 and PI502453-1), which in turn outyielded FFC (Table 1).

In space planted plots, SSC were equivalent to SFC for total yearly yield and first harvest yield (Table 2). In both of these cases, FFC were inferior to both SSC and SFC. Subsequent harvest yield was greatest for SSC, least for FFC, and intermediate for SFC. Of the two falcata parental genotypes, WISFAL-6 produced SFC progeny equivalent to SSC for all yield traits, but PI502453-1 was inferior for total and subsequent harvest yields (Table 1). Note that these space-planted results are a subset of our previous study (Riday & Brummer, 2002a), restricted to the two falcata and three sativa genotypes.

Correlations of entry means between the two planting treatments during first and subsequent harvests showed correlations of $r = 0.55$ and $r = 0.61$, respectively (Table 2). These correlation coefficients compare favorably to that of Pearson and Elling (1961; $r = 0.62$) in their comparison between space planted and sward plots. These results suggest that although inferences of sward plot performance based on space planted plot results should be used cautiously, the correlation coefficients are large enough for the inference to have value to alfalfa breeding programs. The correlation between first and subsequent harvests within plot type are $r = 0.80$ for space plots and $r = 0.69$ for sward plots (Table 2).

We calculated mid-parent heterosis for each of the sativa-falcata hybrid crosses using the SSC and FFC means as the parental values. Sativa-falcata hybrids expressed less heterosis in sward plots than in space planted plots (Table 3). This effect was noted for all

Table 1. Comparisons of entry category means (sativa x sativa crosses, sativa x falcata crosses, falcata x falcata crosses, and check varieties) in space-planted plots and sward plots for total yearly yield, first harvest yield, and subsequent harvest yield for three-year post-establishment year means

	Space-planted plot yield			Sward plot yield		
		Harvest			Harvest	
Entry category	Year total	1st	Sub.	Year total	1st	Sub.
	g $plant^{-1}$			Mg ha^{-1}		
Sativa x sativa crosses	356a[a]	177a	179a	20.6a	7.0a	13.6a
Sativa x falcata crosses	332a	174a	158b	17.9b	7.0a	10.8c
WISFAL-6	365[NS b]	187[NS]	178[NS]	19.6[NS]	7.6*	11.9***
PI502453-1	300***	161[NS]	138***	16.2***	6.4*	9.7***
Falcata x falcata crosses	201c	108b	93c	13.6c	5.9b	7.7d
Check varieties	288b	136b	152b	18.6b	6.7ab	11.8b

*,**, *** contrast between falcata genotype and sativa x sativa crosses is significant at $P = 0.05$, 0.01, and 0.001 levels respectively. NS = not significant.
[a]Cross types or checks within columns with different letters are significantly different at $P = 0.05$.
[b]Comparisons of WISFAL-6 and PI502453-1 with the respective sativa x sativa cross.

Table 2. Correlations of biomass yield and yield heterosis between plot types and between harvests

Correlation	Yield	Yield heterosis
	r	
Space-planted vs. sward plot yield		
First harvest	0.55[NS]	0.37[NS]
Subsequent harvests	0.61*	0.85*
First vs. subsequent harvest yield		
Space-planted plots	0.80**	0.60[NS]
Sward plots	0.69**	0.95**

*, ** significant at $P = 0.05$ and 0.01 levels respectively.

Table 3. Heterosis percentage comparisons between space planted plots and sward plots for falcata genotypes crossed to three sativa tester genotypes, based on three years of post-establishment year heterosis means

	sativa x falcata		Sativa x WISFAL-6		Sativa x PI502453-1	
Yield heterosis	Space	Sward	Space	Sward	Space	Sward
	%					
Year total	20	4**[a]	30	14*	11	−7*
First harvest	26	9*	31	19[NS]	22	0*
Subsequent harvests	15	0**	28	11**	1	−12[NS]
Harvest contrast[b]	*	*	NS	NS	**	NS

*, ** significant at $P = 0.1$ and 0.05 levels respectively; NS = not significant.
[a]Contrast between space planted and sward plots for the respective heterosis measure.
[b]Contrast between first harvest and subsequent harvest heterosis within plot type.

three yield variables (total yearly, first harvest, and subsequent harvest), as well as in both WISFAL-6 and PI502453-1 crosses with sativa. Heterosis declined from first to subsequent harvests (Table 3), supporting our previous results (Riday & Brummer, 2002a, 2003a). Only subsequent harvest yield heterosis was correlated between plot types (Table 2). First and subsequent harvest heterosis was highly correlated in sward plots (Table 2).

Both the more upright WISFAL-6 and the more decumbent PI502453-1 had lower heterosis in sward plots, indicating that a height differential was not necessarily the cause of the heterosis decline. Sativa x falcata crosses have superior regrowth to SSC in the spring after emerging from winter dormancy (Riday & Brummer, 2002b, 2003b). The observation that sativa-falcata heterosis was lower in sward than in space planted plots during first harvest for both falcata genotypes suggests that differential regrowth ability was not the (sole) cause of the decreased heterosis.

The most likely explanation for the decline in heterosis is the more intense interplant competition in the sward that prevents the full expression of a plant's phenotype seen in space planted nurseries. In particular, the SFC plants of the two falcata genotypes were wider than SSC based on previous results in semi-spaced plant conditions (Riday & Brummer, 2003a), and swards likely limited this expression. Similar declines in heterosis between spaced plant and sward plots have been observed in *Lolium perenne* (Foster, 1971). If this explanation is correct, we might expect SSC or FFC crosses to show a similar decline in heterosis, but we could not test that hypothesis with this experiment. Interestingly, however, in space-planted plots, no particular morphological trait was associated with heterosis (Riday & Brummer, 2003b). Denser, taller, and wider plants were associated with increased biomass yield but not with biomass heterosis.

A second reason for the heterosis decline from space-planted to sward plots could be due to the elimination of less vigorous plants. Tysdal & Kiesselbach (1944) mixed various proportions of selfed seed with outcrossed seed and found that actual yields of mixtures with 25% and 50% selfed seed were 10% above theoretical yields. They suggested that in a sward, less vigorous plants were out-competed, leaving more vigorous plants behind, thereby raising yield levels above the theoretical expectation. Other experiments support this result (Veronesi & Lorenzetti, 1983; Viands et al., 1988). In this study, we can imagine intrasubspecific crosses (i.e. SSC and FFC) having more to gain in competitive swards than SFC because the former are more inbred. Relative to space planted plot performance, SSC and FFC in sward plots could receive a yield boost above their noncompetitive space-planted performance because weaker plants will not survive; in contrast, SFC would have fewer weak plants and hence little change would be observed between plot types. This would translate into lower heterosis values in sward plots compared WITH space-planted plots.

This study shows that under a more intensive cutting regime, total yearly yields decline in SFC relative to SSC (Table 1), not due to "lost heterosis," but to a greater proportion of the total yearly yield being derived from harvests subsequent to the first. Subsequent harvests have lower SFC yields and heterosis values (Tables 1, 3; Riday & Brummer, 2002a, 2003a) caused by the slow regrowth of SFC and the negative mid-subspecies heterosis observed for this trait (Riday & Brummer, 2002b, 2003b). This suggests the strong need in falcata population development to select for increased regrowth after harvest in tandem with other traits such as biomass yield and plant height at harvest.

The results of this study should be viewed cautiously. With only two falcata genotypes represented and testing at only one Iowa location, the inference space is quite small. We have previously seen better falcata and SFC performance at Nashua in northeastern Iowa than at Ames, in central Iowa. Given the characteristics of falcata germplasm, we would expect better performance further north. The sward trial was established with a minimum amount of seed, and while the stands were adequate, they were not optimum. Finally, the sward trial was harvested four times per year compared with the three harvest management of the space planted plots. This harvest difference alone likely accounted for some of the variation in heterosis performance for harvests subsequent to the first.

References

Davis, W.E. & J.D.H. Reusch, 1964. The assessment of herbage legume varieties I. Lucerne. J Agric Sci 63: 61–68.

Elliot, F.C., I.J. Johnson & M.H. Schonhorst, 1972. Breeding for forage yield and quality. In: C.H. Hanson (Ed.), Alfalfa Science and Technology, pp. 319–333. ASA, Madison, WI.

Evans, K.H., R.L. Davis & W.E. Nyquist, 1966. Interaction of plant spacing and combining ability in an eight-clone diallel of *Medicago sativa* L. Crop Sci 6: 451–454.

Foster, C.A., 1971. Interpopulational and intervarietal F_1 hybrids in *Lolium perenne*: Heterosis under simulated-sward conditions. J Agric Sci Camb 76: 401–409.

Miller, D.A., J.P. Shrivastava & J.A. Jackobs, 1969. Alfalfa yield components in solid seedings. Crop Sci 9: 440–443.

Pearson, L.C. & L.J. Elling, 1961. Predicting synthetic varietal performance in alfalfa from clonal cross data. Crop Sci 1: 263–266.

Riday, H. & E.C. Brummer, 2005. Heterosis in a broad range of alfalfa germplasm. Crop Sci 45..

Riday, H. & E.C. Brummer, 2004. Morphological variation of *Medicago sativa* subsp. *falcata* genotypes and their hybrid progeny. Euphytica 138: 1–12.

Riday, H. & E.C. Brummer, 2002a. Forage yield heterosis in alfalfa. Crop Sci 42: 716–723.

Riday, H. & E.C. Brummer, 2002b. Heterosis of agronomic traits in alfalfa. Crop Sci 42: 1081–1087.

Rumbaugh, M.D., 1963. Effects of population density on some components of yield of alfalfa. Crop Sci 3: 423–424.

Theurer, J.C. & L.J. Elling, 1964. Comparative performance of diallel crosses and related second-generation synthetics of alfalfa, *Medicago sativa* L. III. Forage yield. Crop Sci 4: 25–28.

Tysdal, H.M. & T.A. Kiesselbach, 1944. Hybrid alfalfa. J Am Soc Agron 36: 649–667.

Tysdal, H.M. & T.A. Kiesselbach, 1939. Alfalfa nursery technic. J Am Soc Agron 31: 83–98.

Veronesi, F. & F. Lorenzetti, 1983. Productivity and survival of alfalfa hybrid and inbred plants under competitive conditions. Crop Sci 23: 577–580.

Viands, D.R., P. Sun & D.K. Barnes, 1988. Pollination control: Mechanical and sterility. In: A.A. Hanson, D.K. Barnes & R.R. Hill (Eds.), Alfalfa and Alfalfa Improvement, pp. 931–960. ASA, Madison, WI.